02/05/16

MEDIA

Marie has arrived at the lab.

Preparation of LB agar following the protocol in the lab and SOB (the pH is nearby 7.5, no need to use Teichmann team's pH meter)

03/05/16

MEDIA

Made LB agar plate using LB agar previously made.

04/05/16

COMPETENT CELLS

Beginning of the preparation for competent cells (E.coli), but the 18°C incubator wasn't available. Manipulation abord. (If we need to borrow some material from other team, we need to ask them the day before at least).

09/05/16

MEDIA

Christian has arrived.

Preparation of LB, LB agar and SOB, but it was contaminated during the night because we haven't access to the autoclave

10/05/16 to 12/05/16

MEDIA

LB, LB agar and SOD are prepared again, and are back from the autoclave in the afternoon.

Beginning to make competent cells using iGEM protocol. Fills all aliquot with 200µL instead of 100µL.

17/05/16

Alexandre has arrived.

Autoclave isn't available

18/05/16

MEDIA

Two 500mL LB agar, 500mL liquid LB, 250mL 2xTY and 1L SOB are made. (Weird things, once the LB agar hardened, it's seem to have much water, moreover some water are on the plate. Maybe too much water or not enough agar. All plates are leave[d] under the hood in order to dry it.

BEGINNING BIOINFORMATCS EXPERIMENTS

(Lab cleanliness test). We pick samples on the table, [table joint]. Three replicate are made for each area (once by each intern) and one more dilution are made from the inoculum.

19/05/16

BACTERIA COMPETENCY TEST

Following iGEM's protocol. Only 3 amounts of DNA were tested (instead of 5): 0.5pg/µL, 5pg/µL, and 50pg/µL. In each tube we added 20µL of competent cells instead of 50µL, heat shock 45 seconds instead of 1 minute, and we used LB instead of SOC. The replicate number 3 was put in LB plate with chloramphenicol add onto it.

Bacteria are put in LB plate in 3 differents concentration, 3 times in sterile area (next to a bec bunsen). The negative control is without plasmid, the positive control is without antibiotic (chloramphenicol). Plates are put into the 37°C incubator at 17h00.

20/05/16

BACTERIA COMPETENCY TEST

09h36, bacteria are competent.

DNA concentration	0,5pg/µL	5pg/µL	50pg/µL
First plate (number of colonies)	8	15	X
Second plate (nb of colonies)	3	16	293
Third plate (nb of colonies)	4	26	521
3 plates mean (nb of colonies)	5	19	407

Using Jean's formula, our cells are competent for 8,07 colonies/µg of DNA.

CLEANLINESS PROTOCOL

48h: only one joint replicate number 2 (dilute=5 , and not dilute=46) had grown colonies.

We puts our dirty fingers into plates, and our clean fingers into other plates.We were three to do that. We puts these plates into the 37°C incubator at 15h00.

23/05/16

Cleanliness test plates with our fingers shown that dirty fingers grows much more colonies than clean fingers. Moreover, it shown that lab cleanliness test plates that shown no colonies was not a problem of manipulation. The lab is clean!

In addition, we put 30µL of competent cells into 30mL LB at 37°C incubator. We will use it tomorrow in order to see how much time bacteria can survive on the desk.

NEW COMPETENT CELLS TEST

in order to have more data and confirm bacteria competent. The transformation was did following iGEM's protocol. Only 3 amounts of DNA were tested (instead of 5): 5pg/µL, 20pg/µL and 50pg/µL. In each tube, 20µL of competent cells were had instead of 50µL, heat-shock during 45sec instead of 1min, used LB instead of SOC and stayed in 37°C incubator during 1h instead of 2h. Growing at 37°C overnight.

Preparation for plasmid loss experimentation. Culture grow at 37°C in 50mL LB from 5 colonies from transformation plate with 5pg DNA.

24/05/16

COMPETENCY TEST

Colony counting

Concentration of DNA (pg/µL)	5	20	50
Replicate 1	2	5	40
Replicate 2	11	21	124
Replicate 3	5	3	76
Mean	6	10	80

There is a problem in our experimentation because there is too much differences between plates from the same tube. Maybe a problem of spawl with bacteria or antibiotic.
So we will try it again tomorrow but this time the antibiotic directly integrated in the LB of the plate.

BACTERIA SURVIVAL TEST IN LABORATORY

setting up the beacon and sowing on the desk competent bacteria. However, sowing equally on the desk isn't possible because of the glass of the desk. Let's see if it work anyway.

PLASMID LOSS

first measure of OD at 600nm / Dilution ½ / Dilution 1/20

regular LB	Antibio : chloro	regular LB	Antibio : chloro	regular LB	Antibio : chloro
tube 1 : 2.903	tube 1 : 3.046	tube 1 : 2.199	tube 1 : 2.410	tube 1 : 0.246	tube 1 : 0.284
tube 3: 2.907	tube 3: 2.978	tube 3: 2.345	tube 3: 2.905	tube 3: 0.290	tube 3: 0.329
tube 4: 3.391	tube 4: 3.017	tube 4: 2.599	tube 4: 2.869	tube 4: 0.336	tube 4: 0.330

Each OD were measured using INNIS team nanodrop in spectrometer mode.

Bring all OD at the same by dilution : (0.284/0.246) * 2ml = -2 → LB to add
Dilution sérielle. 1→ 10000

Goes on plate and in incubator at 37°C overnight

25/05/16

PLASMID LOSS
Some drops had fused (drop 10µL), so need to change to 5µL by drop. There is no difference between LB and LB with chloramphenicol. The pre-culture maybe too concentrate, bacteria are in competition in stationary phase, it doesn't help to loss the plasmid. So, dilution of pre-cultured at 10^{-2}, 10^{-4} et 10^{-6} and grow at 37°C.

BACTERIA SURVIVAL IN LAB
Picking up sample using a sterile cotton swab, for each replicate and control.

COMPETENCY TEST
Competent bacteria transformation with plasmid at the concentration of 5, 20 et 50pg/µL.

26/05/16

BACTERIA SURVIVAL TEST IN LABORATORY
pick up samples from day 2. Place to grow in 37°C incubator.

PLASMID LOSS
retrieval of bacteria from pre-culture cells and place to grow at 37°C incubator.

COMPETENCY TEST
FAIL (maybe due to a too quick heat up of competent cells). Maybe should we made competent cells again.

27/05/16

COMPETENCY TEST: Nothing grow, again...

PLASMID LOSS: prick out colony from PP4, dilute in 2mL, then prick out 100µL and add to grow in 20mL de LB at 37°C incubator.

BACTERIA SURVIVAL IN LAB: pick up samples from day 3. Only control of day 2 grow colonies..

30/05/16

SURVIVAL IN LAB: day 3 incubate during week-end (48h+)

	Day 1	Day 2	Day 3	Day 4	Day 5
Replicate 1	1	0	2080	NE	NE
Replicate 2	0	0	1212	NE	NE
Replicate 3	4	0	1920	NE	NE
Negativ control	0	11	2328	NE	NE

BACTERIA TRANSFORMATION (COMPETENCY TEST): This time, Christian made the transformation with Jean (one experimental for each one). This way, Jean makes sure that we did the experimentation good. If it doesn't work, we will need to make competent cells again...

PLASMID LOSS: grow on plate.

31/05/16

BACTERIA TRANSFORMATION (COMPETENCY TEST): It did work!

25µL competent cells + 150µL lb

	20	50	competency control
jean	171	360	bacteria floor
chris	236	290	bacteria floor

8,5 7.2
11.8 5.8
8.325 *10^3 too low

Cells are competent but not enough.

SURVIVAL IN LAB: day 4 (24h) bacteria in plate 1

PLASMID LOSS: put to grow on antibiotic plate and without antibiotic